MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2020

April–June 2020—Volume 135, pp. 299–308

https://doi.org/10.5248/135.299

Phylogenetic placement of *Acrospeira*

De-Wei Li[1], Rafael F. Castañeda-Ruiz[2], Neil P. Schultes[3]*

[1] *The Connecticut Agricultural Experiment Station, Valley Laboratory, 153 Cook Hill Road, Windsor, CT 06095, USA*

[2] *Instituto de Investigaciones Fundamentales en Agricultura Tropical Alejandro de Humboldt (INIFAT), Académico Titular de la Academia de Ciencias de Cuba, Calle 1 Esq. 2, Santiago de Las Vegas, C. Habana, Cuba, C.P. 17200*

[3] *The Connecticut Agricultural Experiment Station, Department of Plant Pathology & Ecology, 123 Huntington Street, New Haven, CT 06511, USA*

* Correspondence to: *neil.schultes@ct.gov*

Abstract —The morphology of *Acrospeira mirabilis* is described and illustrated based on a collection from Florida, USA. Phylogenies generated from ITS and LSU DNA sequence analyses place the genus *Acrospeira* in *Ceratostomataceae*. Based on molecular data, *Sphaerodes inferior* (≡ *Microthecium retisporum* var. *inferius*) is proposed as *Microthecium inferius* comb. nov.

Key words — asexual fungi, hyphomycete, synanamorph

Introduction

Acrospeira Berk. & Broome was erected and typified by *A. mirabilis* (Berkeley 1857, Berkeley & Broome 1861). Six out of eight species described in this genus have been transferred to other genera (Index Fungorum 2019, MycoBank 2019). With the seventh invalid because it lacks a Latin description (Art. 39.1, Shenzhen Code; Turland & al. 2018), *Acrospeira* has been reduced to its original monotypic status, containing only the type species, *A. mirabilis*. Its current phylogenetic and taxonomic placement is incertae sedis, *Pezizomycotina* (Index Fungorum 2019, MycoBank 2019, Wijayawardene & al. 2018).

A culture of *Acrospeira mirabilis* from a Florida 2018 collection allowed us to conduct a phylogenetic study using two loci (ITS and LSU) and determine its taxonomic placement using both morphological and molecular methods.

Materials & methods

Acrospeira mirabilis was obtained during a March 2018 collection trip in Largo, Florida. The fungus was plated on malt extract medium (MEA) (20 g malt, 20 g agar, 1 L distilled water), purified and subsequently grown at 25°C for 7 days for morphological observation and molecular work. The fungus was mounted in 85% lactic acid for microscopic observation and measurements with a Zeiss Imager. M2 compound microscope using differential interference contrast (DIC) and photographed with a Zeiss Axiocam 506 color camera. Fungal structures were measured using 40–100× objectives and statistically analyzed to determine standard deviations with 95% confidence interval of means.

The fungus culture has been deposited in the UAMH Centre for Global Microfungal Biodiversity at University of Toronto (UAMH), Canada.

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from colonies grown in Petri plates according to the procedure in ZR Fungal/Bacterial DNA MicroPrep Kit. The oligonucleotides ITS5 and LR7 (Vilgalys and Hester 1990, White et al. 1990) were used to amplify a DNA fragment containing the internal transcribed spacer 1 and 2 region (ITS) and a portion of the large subunit rDNA region (LSU) by polymerase chain reaction (PCR) as described in Li & al. (2017). PCR products were purified using QIAquick PCR Purification columns; DNA concentration was determined on a ThermoScientific NanoDrop Lite Spectrophotometer. The purified PCR products were sequenced using oligonucleotides ITS2, ITS3 and ITS5, LROR, LR3R, LR3B, LR5 & LR7 (Vilgalys & Hester 1990, White & al. 1990, Li & al. 2017). The DNA was sequenced at the W.M. Keck Biotechnology Resource Laboratory, Yale School of Medicine (New Haven, CT, USA). New sequence information was deposited in GenBank (Table 1).

The ITS and LSU sequences were used in a BLASTn analysis of GenBank (http://blast.ncbi.nlm.nih.gov). To identify DNA sequences from allied taxa, BLASTn analysis was carried out with exclusion of uncultured/unidentified samples, environmental samples and samples with questionable identifications (Wheeler & al. 2003). Fungal DNA sequences linked to verified fungal voucher cultures that showed significant sequence similarity with the query sequences were chosen for phylogenetic analysis. Additional ITS and LSU sequences from allied taxa were obtained totaling 44 sequences from 32 fungi for phylogenetic analyses (Table 1).

Phylogenetic analysis

ITS and LSU sequences were aligned independently using MUSCLE (Edgar 2004) and manually corrected. The aligned sequences were trimmed and concatenated with FABOX sequence alignment jointer (http://users-birc.au.dk/palle/php/fabox/alignment_joiner.php).

Table 1. *Acrospeira mirabilis* and taxa of allied genera and their sequences used in the phylogenetic analysis.

Taxon	Country	Strain #	ITS	LSU	Reference
Acrospeira mirabilis	USA	UAMH 12078	**MN061697***	**MN061697**	This study
Dactylidispora ellipsospora	Papua New Guinea	NBRC31376 T	LC146749	3137601	NBRC
D. singaporensis	Singapore	ATCC 38286 (NBRC 30865) T	LC146748	AY015629	Zhang & Blackwell 2002
Gonatobotryum parasiticum	Unknown	NBRC9010	901001	901001	NBRC
Harzia acremonioides	USA	NRRL 54328	HQ288051	HQ288051	D.T. Wicklow, ined.
	Unknown	NBRC 5937 (IMI 52642)	593701	593701	NBRC
	Unknown	NBRC 8881	888101	888101	NBRC
	USA	NRRL 54327	HQ698593	HQ698593	D.T. Wicklow, ined.
	Unknown	CBS 101.17	KY623402	KY623403	Schultes & al. 2017
	France	CBS 101.42	KY623405	KY623406	Schultes & al. 2017
H. cameroonensis	Cameroon	CBS 136420 T	KF777163	KF777216	Crous & al. 2013
H. macrospora	Spain	CBS 122807	KY628683	KY628684	Schultes & al. 2017
H. palmara	Unknown	NBRC8861	886101	886101	Schultes & al. 2017
	Indonesia	CBS 158.49	KY623396	KY623397	Schultes & al. 2017
	Turkey	CBS 995.69	KY623399	KY623400	Schultes & al. 2017
H. patula	Canada	CBS 121524	KY628686	KY628687	Schultes & al. 2017
	Canada	CBS 379.88	KY628689	KY628690	Schultes & al. 2017
H. sphaerospora	USA	UAMH 11865	KT221061	KT221062	Li & al. 2016
H. tenella	Romania	CBS 108.78	KY628693	KY628694	Schultes & al. 2017
	Former Yugoslavia	CBS 121.81	KY628696	KY628697	Schultes & al. 2017
H. verrucosa	Unknown	CBS 101.24	KY623414	KY623415	Schultes & al. 2017
	California	CBS 113456	KY628674	KY628675	Schultes & al. 2017
Melanospora damnosa	France	CBS 113681 (NBRC 100318)	10031801	10031801	Marin-Felix & al. 2018

Taxon	Country	Strain #	ITS	LSU	Reference
Me. 'inaequalis'	USA	NBRC 8426	842601	842601	NBRC
Me. kurssanoviana	Russia	NBRC 8098	KP981479	809801	NBRC
Me. mycoparasitica	Canada	SMCD2220 T	FJ748922	FJ748916	Vujanovic & Goh 2009
Me. pascuensis	Chile	IMI 378527 T	AJ011312	—	Stchigel & al. 1999
Me. tiffanyae	Canada	SMCD2222	FJ748921	FJ748915	Vujanovic, ined.
Me. verrucispora	Papua New Guinea	NBRC 31375 T	KP981480	3137501	Marin-Felix & al. 2018
Me. zamiae	Israel	NBRC 32042	3204201	3204202	NBRC
	Unknown	NBRC 7902	790201	790201	NBRC
Microthecium brevirostre	Canada	ATCC 42427	—	AY015627	Zhang & Blackwell 2002
M. ciliatum	Japan	NBRC 9829 T	KP981481	KP981458	NBRC
M. fimbriatum	Unknown	NBRC 8615	861501	861501	NBRC
M. fimicola	Unknown	NBRC 8354	835401	835401	NBRC
	Japan	NBRC 9555	955501	955501	NBRC
M. fusisporum	Unknown	NBRC 8806	880601	880601	NBRC
M. quadrangulare	Spain	CBS 112763 T	KY628699	KY628700	Schultes & al. 2017
M. tenuissimum	Spain	CBS 112764 T	KY628705	KY628706	Schultes & al. 2017
M. zobelii	Unknown	NBRC 9442	944201	KP981476	NBRC
Pseudomicrothecium subterraneum	Unknown	BJTC Fan1001	—	JN247804	Fan & al. 2012
Sphaerodes inferior	Japan	NBRC 8366 T	836601	836601	NBRC
Vittatispora coorgii	India	BICC 7817 T	—	DQ017375	Rehner & Samuels 1995
Xylaria hypoxylon	USA	ATCC 42768	AY327477	U47841	Platas & al. 2004

ATCC = American Type Culture Collection, Manassas, United States; **BICC** = Biocon culture collection, India; **CBS** = The Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; **BJTC** = Culture Collection of Capital Normal University, Beijing, China; **IMI** = IMI Fungarium, the Royal Botanic Gardens, Kew, UK; **NBRC** = the NITE Biological Resource Center, Japan; **NFCCI** = the National Fungal Culture Collection of India; **NRRL** = The Agricultural Research Service Culture Collection, USDA, USA; **SMCD** = the Saskatchewan Microbial Collection and Database, the University of Saskatchewan, Canada; **UAMH** = UAMH Centre for Global Microfungal Biodiversity, University of Toronto, Canada.

*The sequence **accession numbers in bold** indicate that these sequences were sequenced in this study.

The sequence dataset with 44 nucleotide sequences and 1039 positions was phylogenetically analysed using the maximum likelihood procedures, Tamura-Nei model, and uniform rates with MEGA7 (Kumar & al. 2016, Tamura 1992); all sites were treated equally. All sites were equally weighted, and gaps were treated as missing data. A bootstrap was calculated with 1000 replicates and posed statistical support at ≥70%. *Xylaria hypoxylon* ATCC42768 was designated as an outgroup.

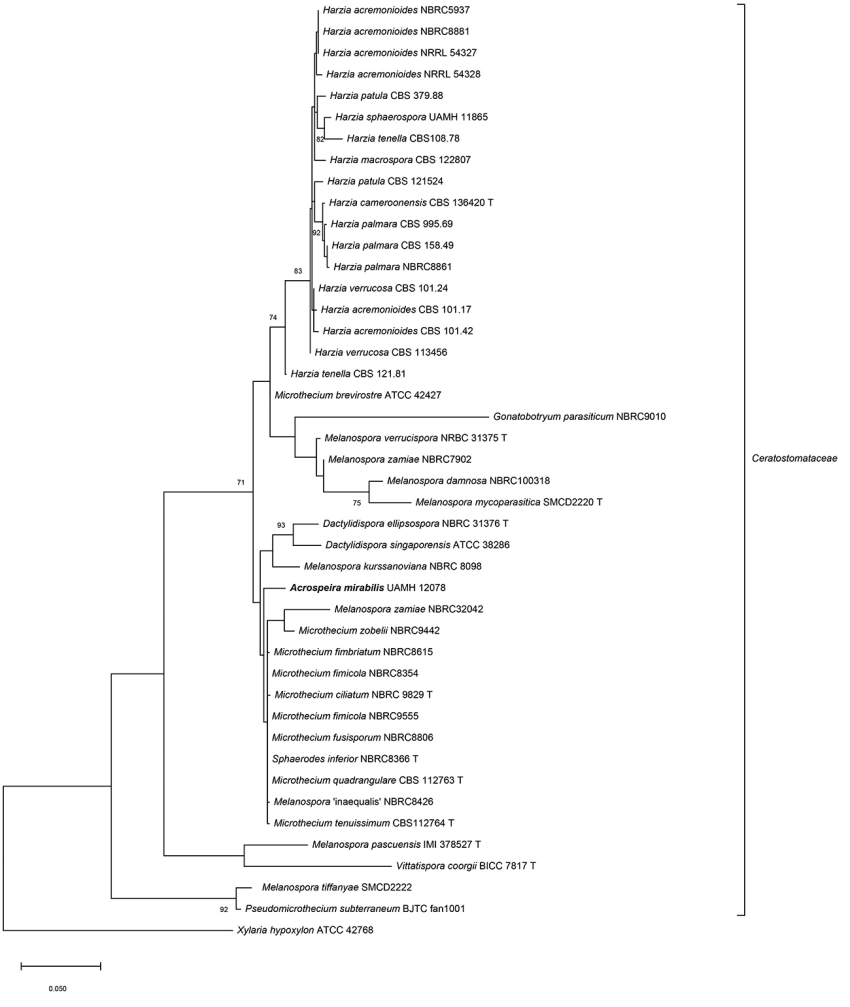

Fig. 1. Maximum Likelihood analysis of *Acrospeira mirabilis* and allied taxa, based on 44 sequences. *Xylaria hypoxylon* ATCC42768 is included as outgroup. The bootstrap test was conducted with 1000 replicates; bootstrap values >70% are indicated at the nodes. The scale bar indicates the number of expected changes per site. Annotations 'T' indicate the ex-type sequences included in the analysis.

Results

The ITS and LSU phylogenetic analyses place *Acrospeira mirabilis* in the same clade as genera in *Ceratostomataceae* (Fig. 1). *Acrospeira mirabilis* defines a sister clade to the clade including *Microthecium zobelii* (the generic type), *M. ciliatum*, *M. fimbriatum*, *M. fimicola*, *M. fusisporum*, *M. quadrangulare*, *M. tenuissimum*, *Sphaerodes inferior*, *Melanospora zamiae* NBRC32042, *Me.* 'inaequalis', and *Me. zamiae* (NBRC32042) (Fig. 1).

Taxonomy

Acrospeira mirabilis Berk. & Broome, Intr. Crypt. Bot.: 305 (1857) Fig. 2

This fungus is dimorphic. Colonies attained 35 mm in 7 days on MEA at 25°C, cottony, chocolate brown. Mycelia immersed or aerial. Hyphae septate, hyaline, smooth, 3–6.8 µm diam.

Dominant state: Conidiophores semi-macronematous, mononematous, terminally or laterally arising from the hyphae and branched, hyaline, 15–35 µm long. Conidiogenous cells monoblastic integrated, terminal, determinate: branch ends enlarged and coiled, hyaline. Conidia solitary, dry, 3-celled, developed by dividing enlarged and coiled portion of conidiogenous cell with 2 transverse septa—the terminal cell enlarged greatly into a (sub)sphere, golden brown, tuberculate, (19.4–)21.9–27.1 (–30.5) µm diam. (mean ± SD = 24.5 ± 2.6 µm, n = 30), thick-walled; the median cell enlarged, smooth, hyaline to golden (paler than terminal cell), tuberculate, hemispherical, or dome-shaped, ornamented; and the basal cell hyaline to yellow and smooth—the whole conidia (23.7–)25.4–30.4(–33.6) × (19.4–)21.9–27.1(–30.5) µm (27.9 ± 2.5 × 24.5 ± 2.6 µm, n = 30), often with a visible germ pore on the enlarged (sub)spherical terminal cell. Conidial secession schizolytic.

Synanamorph: phialidic conidial ontogeny. Conidiophores micronematous, mostly reduced to conidiogenous cells, arranged primarily on aerial hyphae, rarely on assimilative hyphae. Conidiogenous cells monophialidic, discrete, lageniform or ampulliform, rarely integrated, subulate, (5.5–)6.4–11.6(–14.7) × (3.4–)3.6–4.4(–4.9) µm (9.0 ± 2.6 × 4.0 ± 0.4 µm, n = 21), hyaline, smooth. Conidia basipetal, ellipsoid, ovoid, lacrimiform, subglobose or globose, 1-celled, hyaline, smooth, (2.4–)2.7–3.5 (–4.0) × (2–)2.2–2.8(–3.1) µm (3.1 ± 0.4 × 2.5 ± 0.3 µm, n = 30).

Specimens examined: **UNITED STATES**, **Florida**: **Largo**, 27°53′02″N 82°48′31″W, on unknown leaf litter, 3.II.2018, coll. De-Wei Li (UAMH 12078).

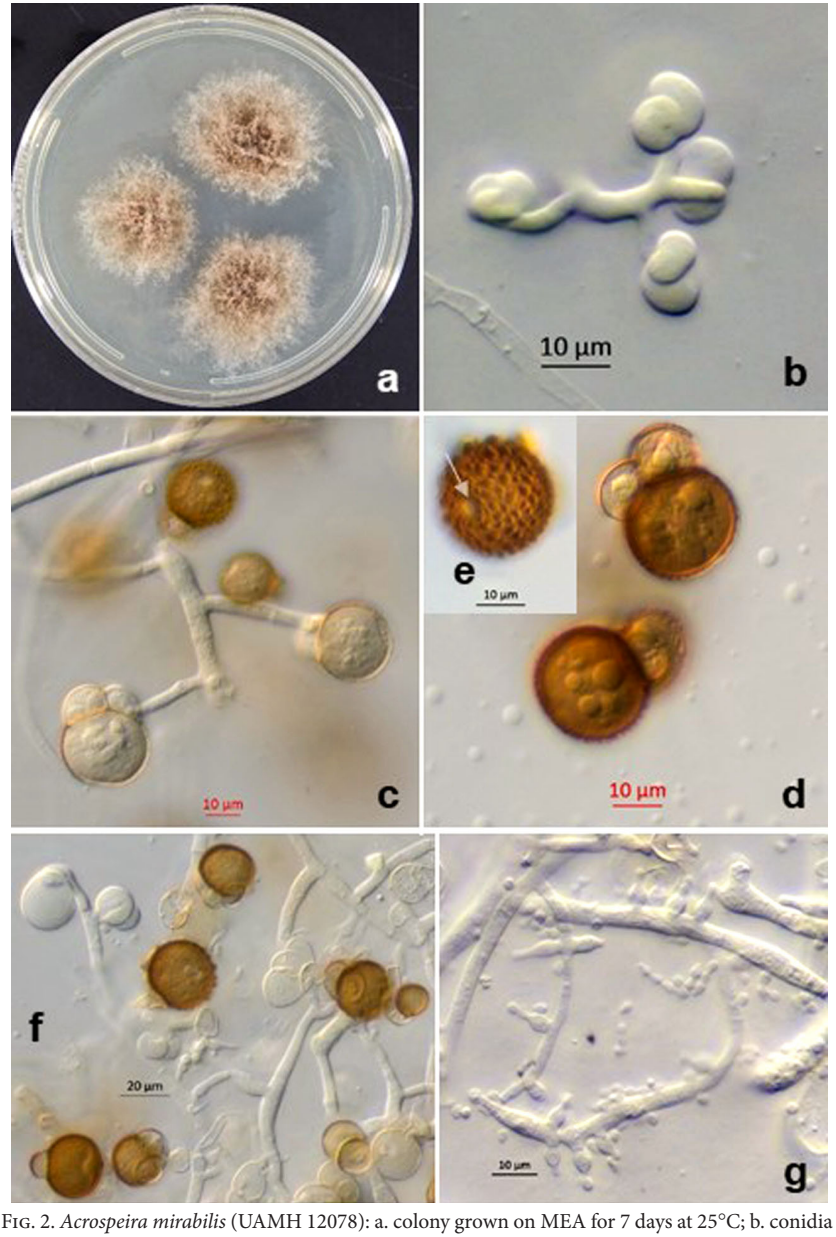

Fig. 2. *Acrospeira mirabilis* (UAMH 12078): a. colony grown on MEA for 7 days at 25°C; b. conidia at coiled stage and conidiophores; c. conidiophores, conidiogenous cells. and conidia; d. conidia; e. surface ornament of conidium with a germ pore (arrowed); f. bimorphic states at different developmental stages; g. synnanamorphic state, conidia and phialides.

Discussion

Conidia of our collection are smaller than those of those described in previous reports (Berkeley 1857, Berkeley & Broome 1861, Ellis 1971). In USA, *A. mirabilis* has previously been reported from Florida, Massachusetts, and Michigan (Donis González & al. 2010, Farr & Rossman 2019).

Acrospeira has a phialidic synanamorph, while *Harzia* Costantin develops a proteophiala synanamorph. The proteophiala-like synanamorph is a key morphological character for merging the formerly accepted genera *Olpitrichum* G.F. Atk. and *Chlamydomyces* Bainier into *Harzia* (supported by phylogenetic analyses) and placing an emended *Harzia* in *Ceratostomataceae* (Li & al. 2016, Schultes & al. 2017). Whether the phialidic synanamorph plays a similar role in *Ceratostomataceae*, it is necessary to conduct further morphological and phylogenetic studies including other anamorphic and related genera. Seifert & al. (2011) suggested that *Acrospeira* belongs to *Ceratostomataceae*, but there is no phylogenetic study or discovery of the sexual state of the type species to support their statement. Our present phylogenetic results support the opinion of Seifert & al. (2011) and firmly demonstrate its taxonomic placement in *Ceratostomataceae*.

Microthecium was resurrected by Marin-Felix & al. (2018) for the M-S (*Melanospora-Sphaerodes*) 2 clade in Schultes & al. (2017). Marin-Felix & al. (2018), who examined the ex-type CBS 994.72 of *Sphaerodes retispora* var. *retispora,* suggested that it was contaminated by a host fungus and that *S. inferior* and *S. retispora* var. *retispora* were conspecific. They demoted *S. inferior* to a synonym of *S. retispora* based on their opinion that there were not enough morphological differences between the two species. However, although Marin-Felix & al. (2018) used the sequences of ex-type NBRC 8366 of *S. inferior* in their phylogenetic analysis, they did not include the sequences of ex-type *S. retispora* var. *retispora*. The sequence from the *S. retispora* ex-type fell into a clade with *Hypocrea schweinitzii* NBRC9063-T and *Trichoderma viridescens* CBS 433.34-T (Schultes & al. 2017), unrelated to *Fusarium* (the host fungus contaminant). We re-examined ex-type (CBS 994.72) of *S. retispora* var. *retispora:* the sexual stage did not develop on MEA, although the monophialidic anamorph was present. The culture was pure and we found no indication that the ex-type was contaminated. In our opinion, the relationship between *S. inferior* and *S. retispora* remains unsettled, and *S. inferior* should be retained as a separate taxon in *Microthecium*.

Microthecium inferius (Udagawa & Cain) D.W. Li, R.F. Castañeda & N.P. Schultes, **comb. nov.**

MB 831435

≡ *Microthecium retisporum* var. *inferius* Udagawa & Cain, Canad. J. Bot. 47(12): 1928. 1970 ["1969"], as "*inferior*".

≡ *Sphaerodes retispora* var. *inferior* (Udagawa & Cain) P.F. Cannon & D. Hawksw., Bot. J. Linn. Soc. 84: 149. 1982.

≡ *Sphaerodes inferior* (Udagawa & Cain) D.W. Li & N.P. Schultes, Fung. Biol. 121(10): 901. 2017.

Our phylogeny places the unpublished *Melanospora* 'inaequalis' NBRC 8366 = TRTC 41540 in the *Microthecium* clade. The conserved strains of *Melanospora* 'inaequalis' need to be studied in the future. Further research of genera in *Ceratostomataceae* is also required, especially *Melanospora* s.l. and *Sphaerodes* s.l., given the polyphyletic characters of their representatives.

Acknowledgments

The authors express their sincere gratitude to Dr. Luis F.P. Gusmão (Universidade Estadual de Feira de Santana, Brazil) and Dr. Patricia Fiuza (Universidade Federal do Rio Grande do Norte, Brazil) for their critical review of the manuscript, and Noelle Strzalkowski and Sumeyra Yavuz for technical assistance. We would like to express our gratitude to Dr. Carole Cheah for providing access to some literature. This work is supported by Hatch Grant [grant no. CONH00813] from the USDA National Institute of Food and Agriculture. Dr. Lorelei Norvell's editorial review and Dr. Shaun Pennycook's nomenclature review are greatly appreciated.

Literature cited

Berkeley MJ. 1857. Introduction to cryptogamic botany. H. Bailliere, London. 604 p. https://doi.org/10.5962/bhl.title.56946

Berkeley MJ, Broome CE. 1861. Notices of British fungi (952-985). Annals and Magazine of Natural History, series 3, 7: 449–456.

Crous PW, Groenewald JZ, Roux J. 2013. Fungal Planet 169 – 26 November 2013. *Harzia cameroonensis* Crous & Jol. Roux, sp. nov. Persoonia 31: 212–213. http://www.fungalplanet.org/content/pdf-files/FungalPlanet169.pdf

Donis González IR, Fulbright DW, Ryser ET, Guyer D. 2010. Shell mold and kernel decay of fresh chestnuts in Michigan. Acta Horticulturae 866: 353–362. https://doi.org/10.17660/ActaHortic.2010.866.45

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. https://doi.org/10.1093/nar/gkh340

Ellis MB. 1971. Dematiaceous hyphomycetes. CABI publishing, Wallingford, UK

Fan L, Hou Cl, Cannon PF, Li Y. 2012. A new species of *Melanospora* on truffles from China. Mycologia 104(6): 1433–1442. https://doi.org/10.3852/11-338

Farr DF, Rossman AY. 2019. Fungal Databases, U.S. National Fungus Collections, ARS, USDA. Retrieved April 2, 2019, from https://nt.ars-grin.gov/fungaldatabases/

Index Fungorum. 2019. Index Fungorum. http://www.indexfungorum.org. Accessed April 2, 2019

Kumar S, Stecher G, Tamura K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33(7): 1870–1874. https://doi.org/10.1093/molbev/msw054

Li DW, Schultes NP, Vossbrinck C. 2016. *Olpitrichum sphaerosporum*: the first record in the USA and its phylogenetic placement. Mycotaxon 131: 123–133. http://dx.doi.org/10.5248/131.123

Li DW, Schultes NP, Chen JY, Wang YX, Castañeda-Ruiz RF. 2017. *Circinotrichum sinense*, a new asexual fungus from Hubei, China. Botany 95(12): 1099–1108. https://doi.org/10.1139/cjb-2017-0132

Marin-Felix Y, Guarro J, Cano-Lira JF, Garcia D, Miller AN, Stchigel AM. 2018. *Melanospora* (*Sordariomycetes*, *Ascomycota*) and its relatives. MycoKeys 44: 81–122. https://doi/org/10.3897/mycokeys.44.29742

MycoBank. 2019. MycoBank (Fungal Databases Nomenclature and Species Banks). Accessed April 2 2019: www.mycobank.org.

Platas G, Ruibal C, Collado J. 2004. Size and sequence heterogeneity in the ITS1 of *Xylaria hypoxylon* isolates. Mycological Research 108(1): 71–75. https://doi.org/10.1017/S0953756203008815

Rehner SA, Samuels GJ. 1995. Molecular systematics of the *Hypocreales*: a teleomorph gene phylogeny and the status of their anamorphs. Canadian Journal of Botany 73(Suppl. 1): S816–S823. https://doi.org/10.1139/b95-327

Schultes NP, Murtishi B, Li DW. 2017. Phylogenetic relationships of *Chlamydomyces*, *Harzia*, *Olpitrichum* and their sexual allies, *Melanospora* and *Sphaerodes*. Fungal Biology 121(10): 890–904. https://doi.org/10.1016/j.funbio.2017.07.004

Seifert KA, Morgan-Jones G, Gams W, Kendrick B. 2011. The genera of hyphomycetes. CBS-KNAW Fungal Biodiversity Centre Utrecht, Utrecht, The Netherlands. 997 p.

Stchigel AM, Cano J, Guarro J. 1999. A new species of *Melanospora* from Easter Island. Mycological Research 103(10): 1305–1308. https://doi.org/10.1017/S095375629900845X

Tamura K. 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. Molecular Biology and Evolution 9(4): 678–687. https://doi.org/10.1093/oxfordjournals.molbev.a040752

Turland NJ, Wiersema JH, Barrie FR, et al., 2018. International code of nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. Koeltz Botanical Books.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172(8): 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Vujanovic V, Goh YK. 2009. *Sphaerodes mycoparasitica* sp. nov., a new biotrophic mycoparasite on *Fusarium avenaceum*, *F. graminearum* and *F. oxysporum*. Mycological Research 113(10): 1172–1180. https://doi.org/10.1016/j.mycres.2009.07.018

Wijayawardene NN, Hyde KD, Lumbsch HT, Liu JK, Maharachchikumbura SS, Ekanayaka AH, Tian Q, Phookamsak R. 2018. Outline of *Ascomycota*: 2017. Fungal Diversity 88: 167–263. https://doi.org/10.1007/s13225-018-0394-8

Wheeler DL, Church DM, Federhen S, Lash AE, Madden TL, Pontius JU, Schuler GD, Schriml LM, Sequeira E, Tatusova TA, Wagner L. 2003. Database resources of the National Center for Biotechnology. Nucleic Acids Research 31(1): 28–33. https://doi.org/10.1093/nar/gkg033

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. 315–322, in: MA Innis MA & al. (eds). PCR protocols: a guide to methods and applications. Academic Press, Inc., New York. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Zhang N, Blackwell M. 2002. Molecular phylogeny of *Melanospora* and similar pyrenomycetous fungi. Mycol. Res. 106: 148–155. https://doi.org/10.1017/S095375620100535